TECHNICAL MANUAL

Aldehyde Dehydrogenase (ALDH) Activity Assay Kit

- **SKU CODE:** MAES0228
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- First measurement at 450 nm
- Incubate at 37°C for 45 minutes
- Second measurement at 450 nm
- Calculate ALDH activity

2. Intended use

This kit can be used to measure aldehyde dehydrogenase (ALDH) activity in serum, plasma, and animal tissue samples.

3. Detection principle

The main pathway of alcohol metabolism involves the oxidation of alcohol by alcohol dehydrogenase (ADH) to acetaldehyde, followed by NADH-dependent acetaldehyde dehydrogenase (ALDH) oxidation to acetic acid.

The detection principle of this kit is based on the substrate being transformed by aldehyde dehydrogenase to convert NAD+ into NADH. Under the action of an electron coupling agent, NADH transfers electrons to WST-8 to produce a yellow product. The activity of ALDH can be calculated by measuring the change in absorbance at 450 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size3 (500Assays)	Storage
Reagent 1	Buffer Solution	9 mL × 1 vial	18 mL × 1 vial	45 mL × 2 vials	-20°C, 12 months
Reagent 2	Coenzyme	Powder × 1 vial	Powder × 2 vials	Powder × 10 vials	-20°C, 12 months, protect from light

Item	Component	Size 1(48 T)	Size 2(96 T)	Size3 (500Assays)	Storage
Reagent 3	Substrate	0.08 mL × 1 vial	0.08 mL × 1 vial	0.2 mL × 2 vials	-20°C, 12 months, protect from light
Reagent 4	Chromogenic Agent	1.2 mL × 1 vial	1.2 mL × 2 vials	6 mL × 2 vials	-20°C, 12 months, protect from light
Reagent 5	Standard	Powder × 1 vial	Powder × 2 vials	Powder × 10 vials	-20°C, 12 months, protect from light
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Incubator, Centrifuge, Microplate reader (440-460 nm, optimum wavelength: 450 nm)

Reagents:

Normal saline (0.9% NaCl), Double distilled water

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of coenzyme stock solution:** Dissolve one vial of coenzyme with 1 mL double distilled water. Store at 2-8°C for 3 days, protected from light.
3. **Preparation of coenzyme working solution:** Before testing, prepare sufficient coenzyme working solution according to the number of test wells. For example, prepare 600 µL of coenzyme working solution by mixing 60 µL of coenzyme stock solution with 540 µL of buffer solution. The coenzyme working solution should be prepared immediately before use and used within 1 hour.
4. **Preparation of substrate working solution:** Before testing, prepare sufficient substrate working solution according to the number of test wells. For example, prepare 300 µL of substrate working solution by mixing 5 µL of substrate with 295 µL of double distilled water. Store at 2-8°C for 3 days, protected from light.

5. **Preparation of reaction working solution:** For each well, prepare 160 µL of reaction working solution by mixing 120 µL of coenzyme working solution with 40 µL of substrate working solution. The reaction working solution should be prepared immediately before use and used within 1 hour.
6. **Preparation of 500 µmol/L standard solution:** Dissolve one vial of standard with 1.6 mL double distilled water. Store at 2-8°C for 3 days, protected from light.
7. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 500 µmol/L standard solution with double distilled water to create a serial concentration. The recommended dilution gradient is: 0, 100, 150, 200, 300, 400, 450, 500 µmol/L.

7. Standard dilution reference

Item	1	2	3	4	5	6	7	8
Concentration (µmol/L)	0	100	150	200	300	400	450	500
500 µmol/L Standard solution (µL)	0	40	60	80	120	160	180	200
Double distilled water (µL)	200	160	140	120	80	40	20	0

8. Sample preparation

1. **Sample preparation:** Serum and plasma: Detect directly. Tissue sample: 1. Harvest the amount of tissue needed for each assay (initial recommendation: 20 mg). 2. Wash tissue in cold normal saline (0.9% NaCl). 3. Homogenize 20 mg tissue in 180 µL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C. 4. Centrifuge at 10,000×g for 15 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection. 5. Meanwhile, determine the protein concentration of supernatant (MAES0177).
2. **Sample dilution:** The recommended dilution factor for different samples is as follows (for reference only): 10% Rat kidney tissue homogenate: 4-8 10% Mouse

kidney tissue homogenate: 4-8 10% Mouse liver tissue homogenate: 4-8 10% Mouse lung tissue homogenate: 4-8 Mouse serum: 4-8 Rat serum: 4-8 Human serum: 4-8 Rat plasma: 4-8 Note: The diluent is normal saline (0.9% NaCl). For dilution of other sample types, please perform a pre-test to confirm the dilution factor.

9. Key points of the assay

1. Avoid bubbles when adding reaction working solution.

2. The reaction process should be performed with protection from light.

10. Operating steps

1. Standard well: Add 20 µL of standard solution with different concentrations to the corresponding wells. Sample well: Add 20 µL of sample to the corresponding wells.

2. Add 160 µL of reaction working solution and 20 µL of chromogenic agent to each well respectively.

3. Mix thoroughly with microplate reader for 3 seconds and stand at room temperature, protected from light, for 5 minutes. Measure the OD value of sample wells at 450 nm with microplate reader, record as A1.

4. Incubate at 37°C for 45 minutes, protected from light. Measure the OD value of sample wells and standard wells at 450 nm with microplate reader, record as A2. Calculate ΔA = A2 - A1. (Note: There is no change in OD value of standard wells; plot the standard curve with the OD value of A2 for standards).

11. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using the absolute OD value of standards as the y-axis and corresponding concentration as the x-axis. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

1. Serum (plasma) sample:

Definition: The amount of ALDH in 1 L liquid sample per 1 minute that hydrolyzes acetaldehyde to produce 1 μmol NADH at 37°C is defined as 1 unit.

ALDH activity (U/L) = (ΔA_450 - b) ÷ a ÷ T × f

2. Tissue sample:

Definition: The amount of ALDH in 1 g tissue protein per 1 minute that hydrolyzes acetaldehyde to produce 1 μmol NADH at 37°C is defined as 1 unit.

ALDH activity (U/gprot) = (ΔA_450 - b) ÷ a ÷ T ÷ C_{pr} × f

[Note]

ΔA_450: The change in OD values of sample well (A_2-A_1).

T: The time of incubation reaction, 45 min.

C_{pr}: The concentration of protein in tissue, gprot/L.

f: Dilution factor of sample before test.

12. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision Data

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.56	2.7	8.50
%CV	3.5	3.0	2.5

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision Data

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.56	2.7	8.50
%CV	6.8	7.5	7.6

Recovery

Three samples of high, medium, and low concentrations were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 99%.

Recovery Data

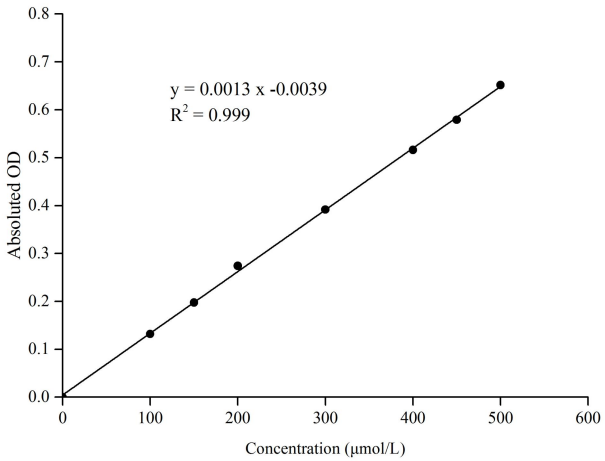
Parameters	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	135	264	425
Observed Conc. (µmol/L)	136.4	253.4	425.0
Recovery rate (%)	101	96	100

Sensitivity

The analytical sensitivity of the assay is 0.01 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (μmol/L)	OD value 1	OD value 2	Average OD	Absolute OD
0	0.056	0.052	0.054	0.000
100	0.184	0.188	0.186	0.132
150	0.253	0.25	0.252	0.198
200	0.333	0.323	0.328	0.274
300	0.459	0.432	0.446	0.392
400	0.576	0.564	0.570	0.516
450	0.633	0.633	0.633	0.579
500	0.708	0.703	0.706	0.652

13. Appendix II Example Analysis

Example analysis:

For mouse liver tissue, take 20 μL of 10% mouse liver tissue homogenate, dilute 4-fold, and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.0013x - 0.0039

OD value of sample A_1: 0.131

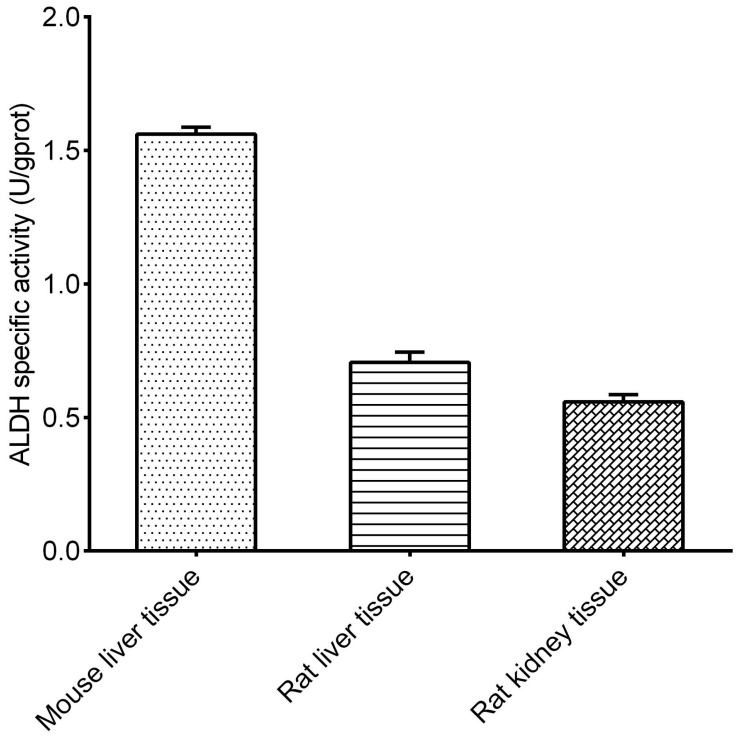
OD value of sample A_2: 0.285

Protein concentration in sample: 7.03 gprot/L

Calculation result:

ALDH activity (U/gprot) = (0.285 – 0.131 + 0.0039) ÷ 0.0013 ÷ 45 ÷ 7.03 × 4 = 1.53 U/gprot

Detection of 10% mouse liver tissue homogenate (protein concentration: 7.03 gprot/L, diluted 4-fold), 10% rat liver tissue homogenate (protein concentration: 5.65 gprot/L, diluted 4-fold), and 10% rat kidney tissue homogenate (protein concentration: 7.35 gprot/L, diluted 4-fold) according to the protocol yielded the following results:



14. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.